Structure Determination of Proline Substitutions in the DNA-Binding Domain of the Yeast Heat Shock Transcription Factor

J. Hardy and H. Nelson (U. of Pennsylvania)

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Introduction: The second helix of the DNA-binding domain of the heat shock transcription factor (HSF) has a proline-centered kink. This proline is conserved in all HSFs. We determined the structure of two proline substituted substitutions and compared them to the wild-type structure in order to determine if the presence of the proline was required to maintain the kink.

Methods and Materials: The DNA-binding domains were expressed and purified by standard methods. Crystals grew in 22-30% PEG 4K, 50-200 mM ammonium acetate, and 100 mM Citrate buffer (pH 5.6 to 6.2). Crystals were frozen prior to data collection. The structures were solved by molecular replacement using aMoRe (Navaza, 1992) and refined using O and CNS (Jones *et al*., 1991, Brunger *et al*.,1998). Details can be found in Hardy, 2000.

Results: Substitution of the proline with either alanine or lysine does not affect the structure of the DNA-binding domain or the degree of bending at the kink (**Figure 1**).

Conclusions: The proline in the α-helical kink of the DNA-binding domain of HSF is not required to maintain the structure of the kink. Additional non-crystallographic experiments showed that the proline is not required for function or stability of the protein. Instead, we found that it is required for the folding kinetics. This suggests that the presence of a proline in a helical kink is important for the folding pathway of a protein and may explain why the proline is conserved in all HSF DNA-binding domains.

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References: A. Brunger *et al*., "Crystallography and NMR system: A new software suite for macromolecular structure determination." Acta. Cryst. D54, 905,1998; J. Hardy, "Role of an alpha-helical bulge and kink in the heat shock transcription factor," Ph.D. Thesis, University of California, Berkeley, 2000; T. Jones *et al*., "Improved methods for binding protein models in electron density maps and the location of errors in these models." Acta. Cryst. A47, 110, 1991; J. Navaza, "aMoRe - an automated package for molecular replacement," Acta Cryst. A50, 157, 1994.

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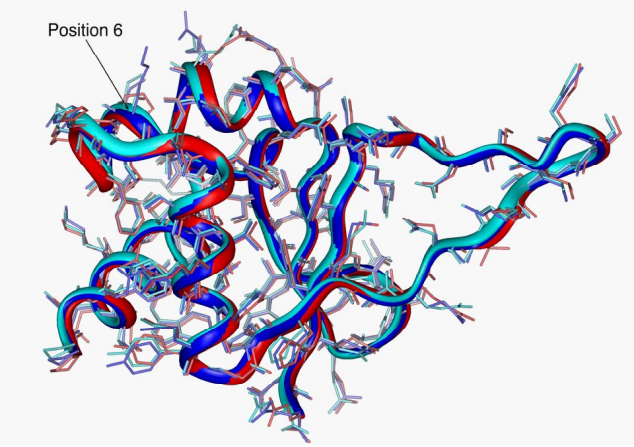

Figure 1. The superpositions of wild-type (red), P6K (blue) and P6A (cyan) DNA-binding domain structures. The position of the proline, which is the sixth position of the second helix, is indicated.